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Improved method for rapid purification of protein kinase from

Janecek J, Dobrova Z, Moravec V, Naprstek J.

streptomycetes.

☐ 1: J Biochem Biophys Methods. 1996 Jan 11;31(1-2):9-15.

Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic.

Protein kinase from Streptomyces lincolnensis was purified nearly to homogeneity using a high performance liquid chromatography (HPLC) and a Pharmacia FPLC system. The procedure used employed column chromatography on DE-53, followed by FPLC affinity chromatography with serine- or threonine-Sepharose (prepared as described in this paper) and gel filtration using a Superose 12 or TSK G3000SW column. Starting with 3.5 g of mycelial proteins, approximately 1 mg of pure enzyme was obtained. The procedure is simple and highly reproducible. The protein kinase thus obtained was nearly pure by silver staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified protein kinase phosphorylated substrate proteins at the seryl residues.

PMID: 8926341 [PubMed - indexed for MEDLINE]

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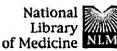
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		□1: Anal Bio	☐ 1: Anal Biochem. 1990 Jun;187(2):205-11.						Related Articles, Link		

Entrez PubMed Overview A high-yield method for the isolation of hydrophobic proteins Help | FAQ and peptides from polyacrylamide gels for protein sequencing. Tutorial New/Noteworthy

Feick RG, Shiozawa JA.

Max-Planck Institut fur Biochemie, Martinsried, Bundesrepublik Deutschland.

A methodological approach is described which allows the isolation of hydrophobic and hydrophilic proteins and peptides in high yield. The technique consists of (1) preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, (2) protein elution from polyacrylamide gels with an organic solvent mixture composed of formic acid/acetonitrile/isopropanol/H2O (50/25/15/10, v/v/v/v), and (3) purification of eluted proteins by size exclusion chromatography on a Superose 12 column using this organic solvent mixture as eluant. The efficiency of this technique was tested with radioactively labeled polypeptides. These proteins were reaction center from Chloroflexus aurantiacus, bacteriorhodopsin, halorhodopsin from Halobacterium halobium, bovine serum albumin, ovalbumin, alpha-chymotrypsinogen A, and cytochrome c. The elution recoveries from polyacrylamide gels were 77-95%; the final yield after chromatographic purification was still 67-76% (with one exception). Subsequent amino acid sequencing was possible without further sample treatment. The sensitivity of the method described was found to be at least 20-30 micrograms protein.

PMID: 2382824 [PubMed - indexed for MEDLINE]

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